Abstract: European Molecular Biology Organization (EMBO) congress, Nice, France (August 2012)

Reliability of pre-implantation genetic diagnosis in a heteroplasmic mitochondrial mouse model

Jitesh Neupane[1], Mado Vandewoestyne[2], Bjorn Heindryckx[1], Sabitri Ghimire[1], Yuechao Lu[1], Sylvie Lierman[1], Chen Qian[1], Dieter Deforce[2], Petra De Sutter[1]

[1]Department for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

[2]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium

Background

Mitochondrial DNA (mtDNA) mutation disorders are a group of diseases that are transmitted exclusively through the maternal line. Most pathogenic mtDNA mutations are present in a heteroplasmic form containing both wild type and mutated mtDNA. In the absence of potential cure, pre-implantation genetic diagnosis (PGD) is expected to diagnose the level of heteroplasmy and prevent transmission of such disorders. The aim of this study was to test the reliability of PGD in a heteroplasmic mouse model containing mtDNA genotypes from BALB and NZB mice.

Observations

First polar bodies (PB) were biopsied from metaphase II (MII) oocytes, which were fertilized by intra-cytoplasmic sperm injection to analyse second PB and zygotes. Zygotes were further cultured to harvest blastomeres from 2, 4 and 8-cell embryos. Heteroplasmic load was measured by restriction fragment length polymorphism method in 162 samples. Results were analysed by Wilcoxon Signed Rank test (significant at $p<0.05$) and Pearson's correlation test (r). No significant difference was seen in levels of heteroplasmy between the first PB (n=10) and ooplasm of MII oocytes (n=10) (r=0.92); between first PB (n=10), second PB (n=10) and zygotes (n=10) (r=0.91 and 0.92 respectively); between first and second PBs (r=0.82); between first PB (n=19), second PB (n=19) and blastomeres (n=74) (r=0.89 and 0.90 respectively); and also among blastomeres (r=0.96). The difference in heteroplasmic load ranged from 0.09 to 15% between MII oocytes and first PBs; 0.34 to 20.85% between first and second PBs; 0.16 to 8.81% between zygotes and first PBs; 0.29 to 8.82% between zygotes and second PBs, 0.03 to 15.17% between first PB and blastomeres; and 0.06 to 16.35% between second PB and blastomeres. The inter-blastomere variation ranged from 0.00 to 12.10%.

Conclusions

Blastomeres are more reliable predictors of heteroplasmic load than the first and/or second PBs. Therefore, blastomere biopsy can be an option for PGD. However, intra-blastomere variation necessitates the biopsy of two blastomeres for accurate diagnosis, which may hamper further embryonic development.